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T7 PROMOTER PRIMER

CTAATACGACTCACTATAGGG
CTAATACGACTCACTATAGGG
GATTATGCTGAGTGATATCCC

cK XbaI PRIMER

CTGCAGGTCGACTGTAGAGGATCTACTAGT
CATGCCTGCAGGTCGACTCTAGAGGATCTACTAGT
GTACGGACGTCCAGCTGAGATCTCCTAGATGATCA

MUTAGENIC SITE XbaI

MUTAGENIC SITE

TTCTGTGCTCTATGGTACAGCAACCTCTGGGTATTCCGT
AAGACACGAGATACCATGTCGTTGGAGACCCATAAGCCA

CACGAGATACCATGACGTTGGAGACCCATA

S95C PRIMER

BamHI

CGTCGTGACTGGGAAAACC
GCAGCACTGACCCTTTTGG
GCAGCACTGACCCTTTTGG

pT7VICHA255

U-19 PRIMER

1. PCR REACTION WITH T7 PRIMER AND S95C
2. PCR REACTION WITH KXbaI PRIMER AND S95C
3. MIX PRODUCTS FROM EACH AT 95°C/10 MIN COOL TO 55°C

BamHI

4. FILL IN WITH POLYMERASE AND NUCLEOTIDES

BamHI

XbaI

5. AMPLIFY WITH PCR

BamHI

AND

BamHI

XbaI

FIG. 1.

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CTA	CAA	CTG	AAT	AGT	CTG	AGG	TCT	GAG	GAC	ACG	GCC	TTG	TAT	TTC	TGT	GCA	AGT	CAT	CGG
GAT	GTT	GAC	TTA	TCA	GAC	TCC	AGA	CTC	CTG	TGC	CGG	AAC	ATA	AAG	ACA	CGT	TCA	GTA	GCC
Leu	Gln	Leu	Leu	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Leu	Tyr	Phe	Cys	Ala	Ser	His	Arg
CDR3																			

TTT	GTT	CAC	TGG	GGC	CAC	GGG	ACT	CTG	GTC	ACT	GTC	GTC	ACT	GTC	TCT	GCA	GCC	AAA	ACG	ACA	CCC	CCA
AAA	CAA	GTG	ACC	CCG	GTG	CCC	TGA	GAC	CAG	TGA	CAG	AGA	CGT	CGG	CGG	CGG	TTT	TGC	TGT	GGG	GGT	
Phe	Val	His	Trp	Gly	His	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala	Ala	Lys	Thr	Thr	Pro	Pro			

FR4

CH1

CCC GGG GAG G

L^{Apal}

FIG. 2B.

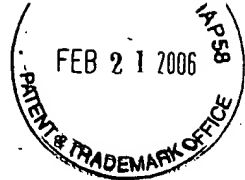


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SstI
CTCAGAGCTC
GCT GTT GTG ACT CAG GAA TCT
GCT GTT GTG ACT CAG GAA TCT
CGA CAA CAC TGA GTC CTT AGA
Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr Leu Thr
FR1
TGT CGC TCA AGT ATT GGG GCT GTT ACA ACT AGT AAC TAT GCC AAC TGG GTC CAA GAA AAA
ACA GCG AGT TCA TAA CCC CGA CAA TGT TGA TCA TTG ATA CGG TTG ACC CAG GTT CTT TTT
Cys Arg Ser Ser Ile Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys
CDR1
CCA GAT CAT TTA TTC ACT GGT GGT CTA ATA GGT GGT ACC AAT AAC CGG GCT CCG GGT GTT CCT
GGT CTA GTA AAT AAG TGA CCA GAT TAT CCA CCA TGG TTA TTG GCC CGA GGC CCA CAA GGA
Pro Asp His Leu Phe Thr Gly Thr Gly Ile Ile Gly Thr Asn Asn Arg Ala Pro Gly Val Pro
CDR2
GCC AGA TTC TCA GGC TCC CTG ATT GGA GAC AAG GCT GCC CTC ACC ATC ACA GGG GCA CAG
CGG TCT AAG AGT CCG AGG GAC TAA CCT CTG TTC CGA CGG GAG TGG TAG TGT CCC CGT GTC
Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln
FR3

FIG. 3A.



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ACT GAA GAT GAG GCA AGA TAT TTC TGT GCT CTA TCG TAC TGC AAC CTC TGG GTG TTC GGT	
TGA CTT CTA CTC CGT TCT ATA AAG ACA CGA GAT ACC ATG ACG TTG GAG ACC CAC AAG CCA	
Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala	Leu Trp Tyr Cys Asn Leu Trp Val Phe Gly
	FR4

CDR3

GGA GGA ACC AAA CTG ACT GTC CTA AGC CAG CCC AAG TCT TCG CCA TCA GTC ACC CTG TTT	
CCT CCT TGG TTT GAC TGA CAG GAT TCG GTC GGG TTC AGA AGC GGT AGT CAG TGG GAC AAA	
Gly Gly Thr Lys Leu Thr Val Leu Ser	Gln Pro Lys Ser Ser Pro Ser Val Thr Leu Phe
TTT GAC TGA CAG GAT TCG	

BsiWI

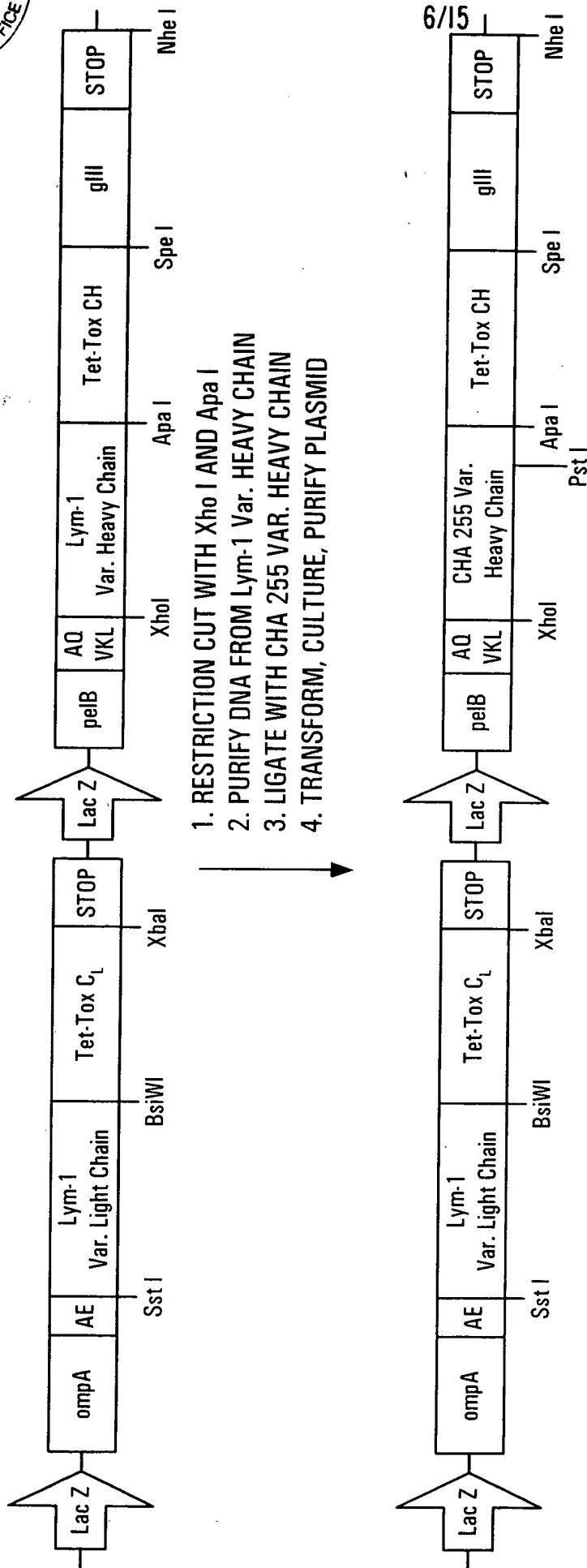
CGTACGCTC

CCG CCC TCC TCT GAA GAG CTA AGC TTG GGA ATC GGA TTC CCG GG	
GGC GGG AGG AGA CTT CTC GAT TCG AAC CCT TAG CCT AAG GGC CC	
Pro Pro Ser Ser Glu Glu Leu Ser Leu Gly Ile Gly Phe Pro Gly	

FR4

CH1

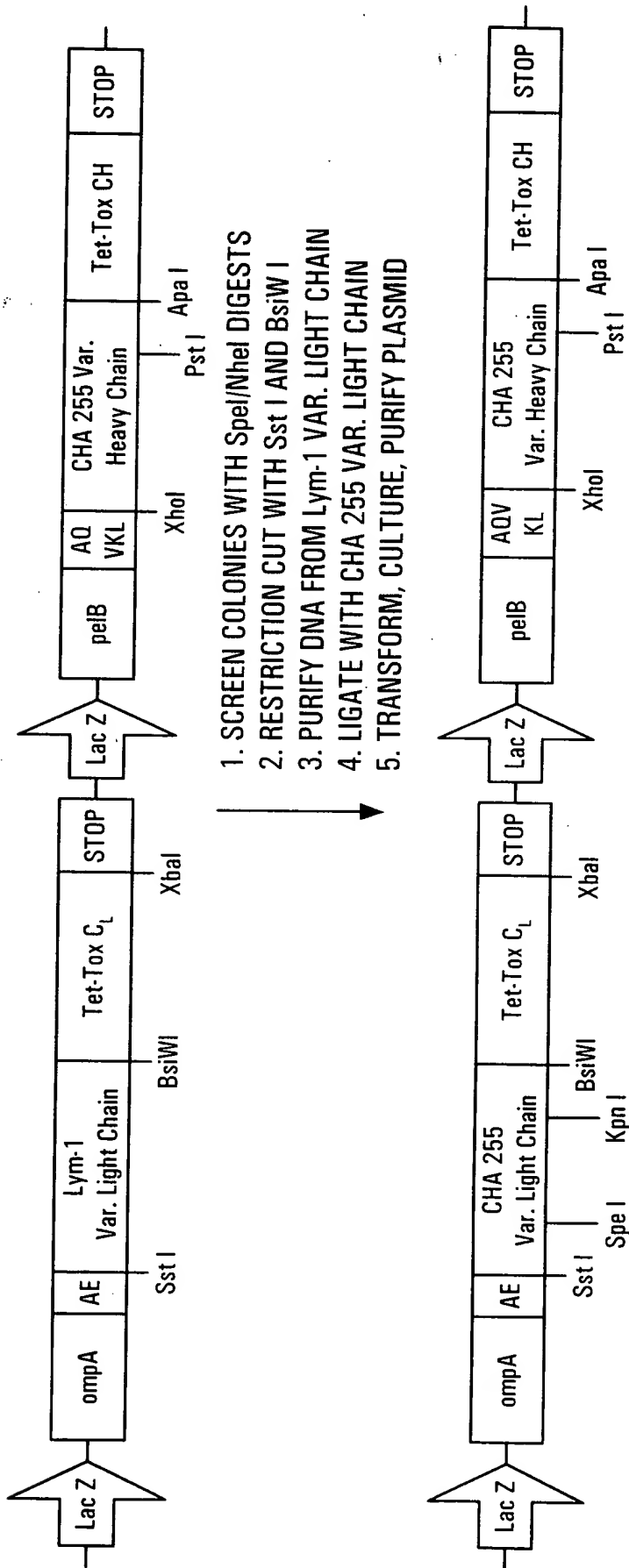
FIG. 3B.



1. SCREEN COLONIES WITH XhoI/PstI DIGESTS
2. SEQUENCE WITH HCF/GX PRIMERS
3. DIGEST POSITIVE COLONY WITH SpeI/NheI
4. LIGATE, TRANSFORM, CULTURE, PURIFY PLASMID

FIG. 4A.

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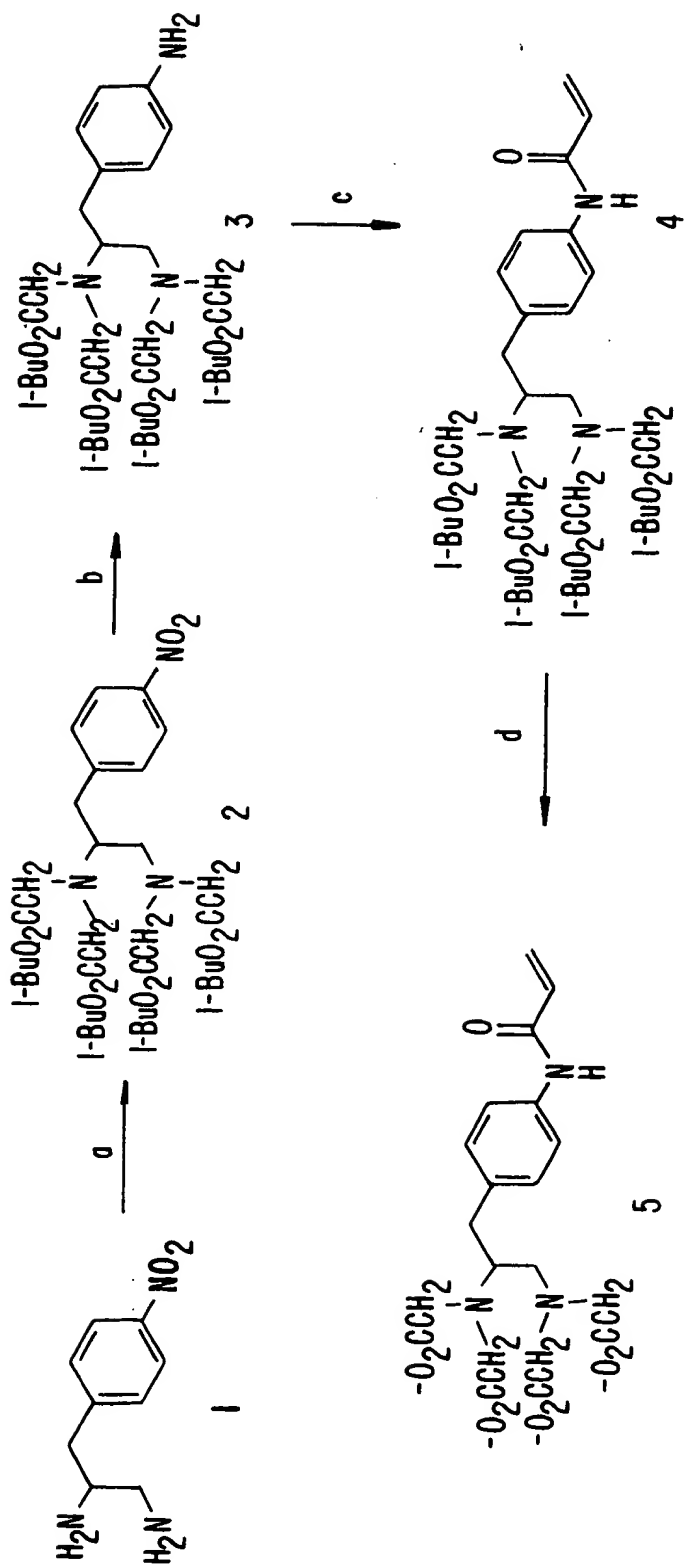


1. SCREEN COLONIES WITH KpnI/XbaI DIGESTS
2. SEQUENCE POSITIVE CLONES WITH Lcf/Kx AND Hcf/Gx
3. EXPRESS PROTEIN AND CHARACTERIZE

FIG. 4B.

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a. $\text{BrCH}_2\text{CO}_2\text{t-Bu}$, DIPEA, KI, DMF; b. H_2 , Pd/C, MeOH; c. acryloyl chloride, DIPEA, CH_2Cl_2 ; d. TFA

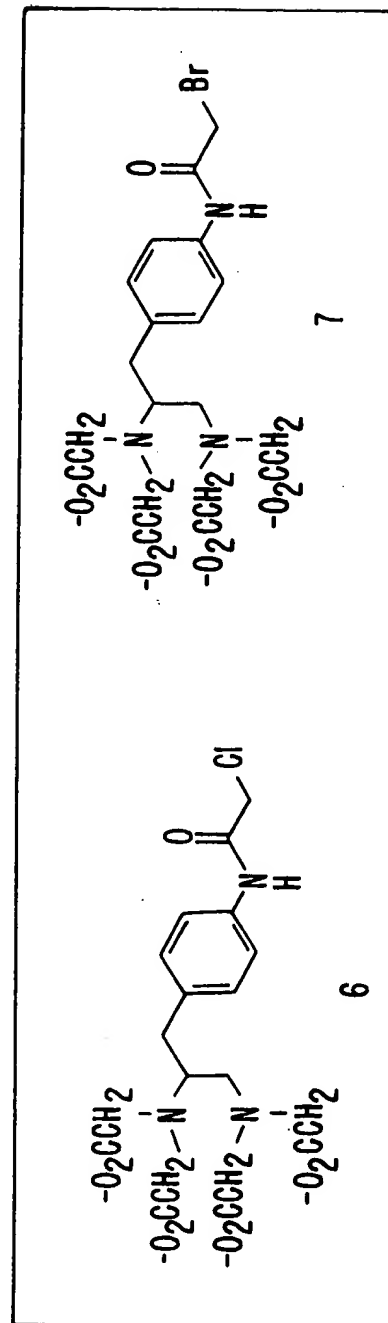
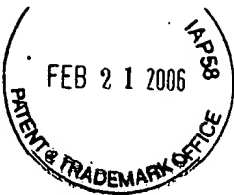


FIG. 5.



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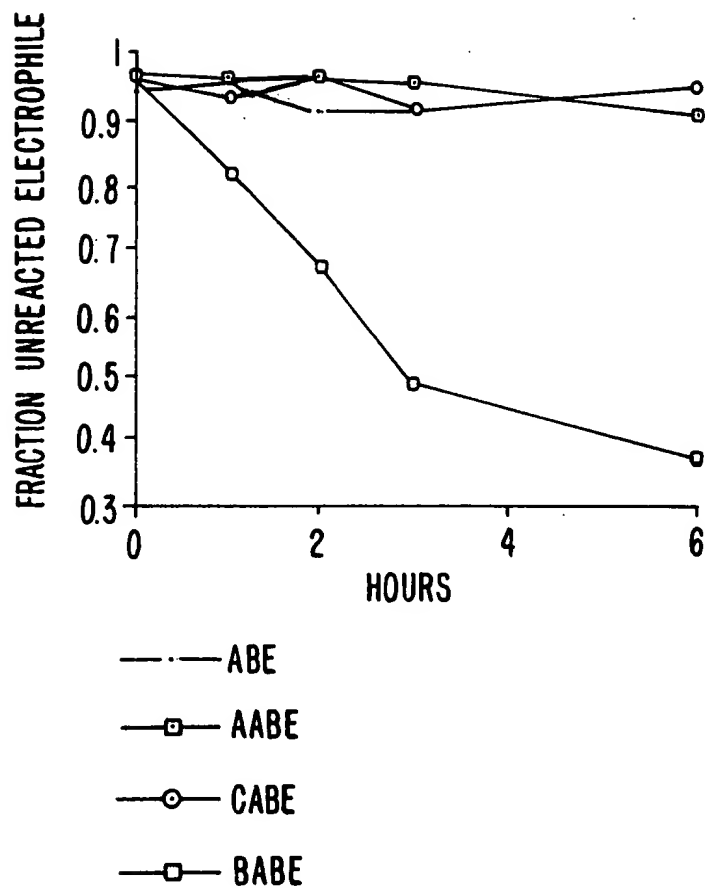


FIG. 6.

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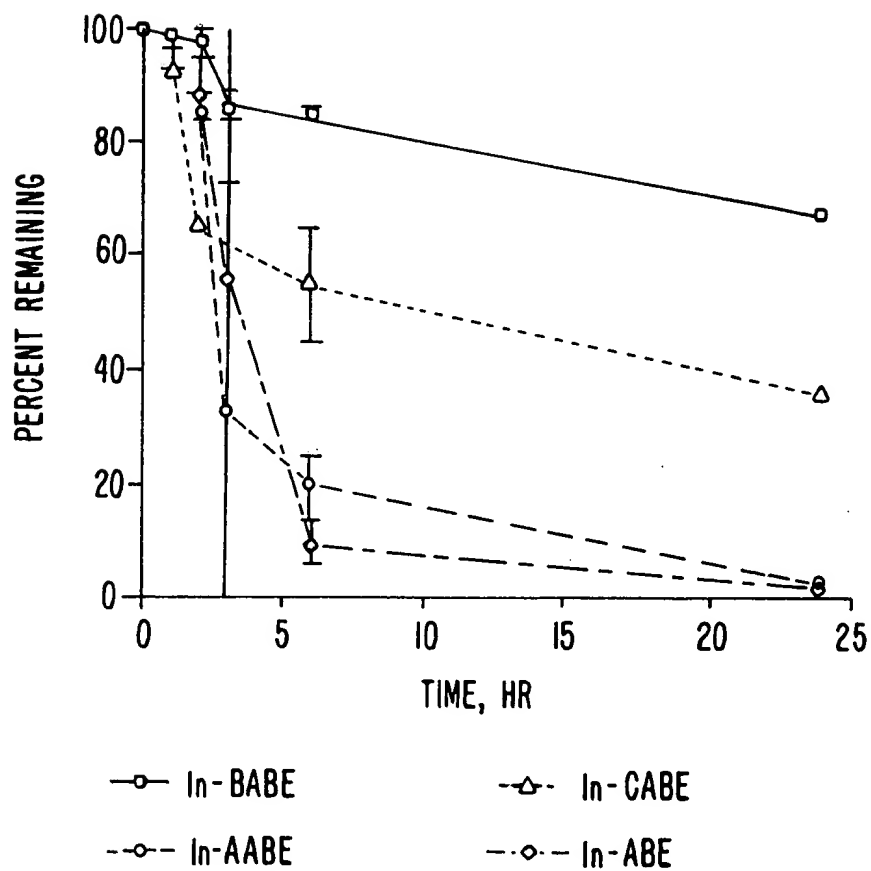


FIG. 7.

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AGATCTGAAGTGACGCTGGTGGAGTCTGGGGGAGACTCAGTGAAGCCTGGAGGGTC
CCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTAAGTGGTGAAACCATGTCTTG
GGTTCGCCAGACTCCGGAGAAGAGGCTGGAGTGGGTGCAACCACTCTTAGTGGTG
GTGGTTTCACCTTCTATTTCAGCCAGTGTGAAGGGTCGTTTCACCATCTCCAGAGACA
ATGCCCAGAACAACCTCTATCTACAACCTGAATAGTCTGAGGTCTGAGGACACGGCCT
TGTATTTCTGTGCAAGTCATCGGTTTGTTCACTGGGGCCACGGGACTCTGGTCACTG
TCTCTGCAGCCAAAACGAOACCCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGA
GCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAAC
CGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCCG
CTGTCTACAGTCTCAAGACTCTACTTCCTCAGCAGCGTGGTGACCGTGCCCTTCA
ACAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC
AAGGTGGACAAGAAAGCAGAGCCCAAATCTTGTGACAAATCTAGAGGGCCCTTCGA
AGGTAAGCCTATCCCTAACCCCTCTCCTCGGTCTCGATTCTACGCGTACCGGTCATCA
TCACCATCACCATTGA

FIG. 8.

AGATCTGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGAAACAGTC
ACACTCACTTGTCGCTCAAGTATTGGGGCTGTTACAACTAGTAAGTATGCCAACTGG
GTCCAAGAAAAACCAGATCATTTATTCACTGGTCTAATAGGTGGTACCAATAACCGG
GCTCCGGGTGTTCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTC
ACCATCACAGGGGCACAGACTGAAGATGAGGCAAGATATTTCTGTGCTCTATGGTA
CTCCTGCCTCTGGGTRTTCGGTGGAGGAACCAAAGTACTGTCCTAAGCCGWACKGT
GGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAC
TGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG
GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGG
ACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGAC
TACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGYTYGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

FIG. 9.

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AGATCTGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGAAACAGTC
 AACTCACTTGTCGCTCAAGTATTGGGGCTGTTACAACTAGTAAGTATGCCAACTGG
 GTCCAAGAAAAACCAGATCATTTATTCAGTGGTCTAATAGGTGGTACCAATAACCGG
 GCTCCGGGTGTTCTGCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTC
 ACCATCACAGGGGACAGACTGAAGATGAGGCAAGATATTTCTGTGCTCTATGGTA
 CTCCAACCTCTGGGTGTTCTGGTGGAGGAACCAAAGTACTGTCCTAAGCCAGCCCA
 AGTCTTCGCCATCAGTCACCCTGTTTCCGCCCTCCTCTGAAGAGCTAAGCTTGGGAA
 TCGGATTGCCGGGGTGCCTGCTGAATAACTTCTATCCCAGAGAGGGCCAAAGTACAGT
 GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAG
 GACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGA
 CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGYTYGC
 CCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

FIG. 10.

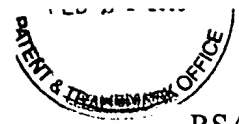
AGATCTGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGAAACAGTC
 AACTCACTTGTCGCTCAAGTATTGGGGCTGTTACAACTAGTAAGTATGCCAACTGG
 GTCCAAGAAAAACCAGATCATTTATTCAGTGGTCTAATAGGTGGTACCAATAACCGG
 GCTCCGGGTGTTCTGCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTC
 ACCATCACAGGGGACAGACTGAAGATGAGGCAAGATATTTCTGTGCTCTATGGTA
 CTCCAACCTCTGGGTGTTCTGGTGGAGGAACCAAAGTACTGTCCTAAGCCAGCCCA
 AGTCTTCGCCATCAGTCACCCTGTTTCCGCCCTCCTCTGAAGAGCTAAGCTTGGGAA
 TCGGATTCCCAGGGGTGCCTGCTGAATAACTTCTATCCCAGAGAGGGCCAAAGTACAGT
 GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAG
 GACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGA
 CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGYTYGC
 CCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

FIG. 11.

RSAVVTQESALTTSPGETVTLTCRSSIGAVTTSNYANWVQEKPDHLFTGLIGGTNNR
 APGVPARFSGSLIGDKAALTITGAQTEDEARYFCALWYSLWVFGGGTKLTVLSRTV
 AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
 SKDSTYLSLSTLTLSKADYEKHKVYACEVTHQGLSLXPVTKSFNRGEC

FIG. 12.

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RSVVVTQESALTTSPGETVTLTCRSSIGAVTTSNYANWVQEKPDHLFTGLIGGTNNR
APGVPARFSGSLIGDKAALTITGAQTEDEARYFCALWYSNLWVFGGGTKLTVLSRTV
AAPSVMFPPSDEQLKSGTASVVCLLNNFYPPREKQVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSXPVTKSFNRGEC

FIG. 13.

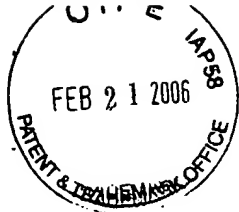
RSVVVTQESALTTSPGETVTLTCRSSIGAVTTSNYANWVQEKPDHLFTGLIGGTNNR
APGVPARFSGSLIGDKAALTITGAQTEDEARYFCALWYCNLWVFGGGTKLTVLSRTV
AAPSVMFPPSDEQLKSGTASVVCLLNNFYPPREKQVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSXPVTKSFNRGEC

FIG. 14.

RSEVTLVEGRGDSVKPGGSLKLSCAASGFTLSGETMSWVRQTPEKRLEWVATTLSGG
GFTFYASAVKGRFTISRDN
AQNNLYLQLNSLRSEDALYFCASHRFVHWGHGTLTVSAAKTPPSVFPLAPSSKS
TSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSRLYFLSSVTVPFNSLGTQTYICNVNHKPSNTK
VDKKAEPKSCDKSRGPFEG
KPIPNLLGLDSTRTGHHHHHH

FIG. 15.

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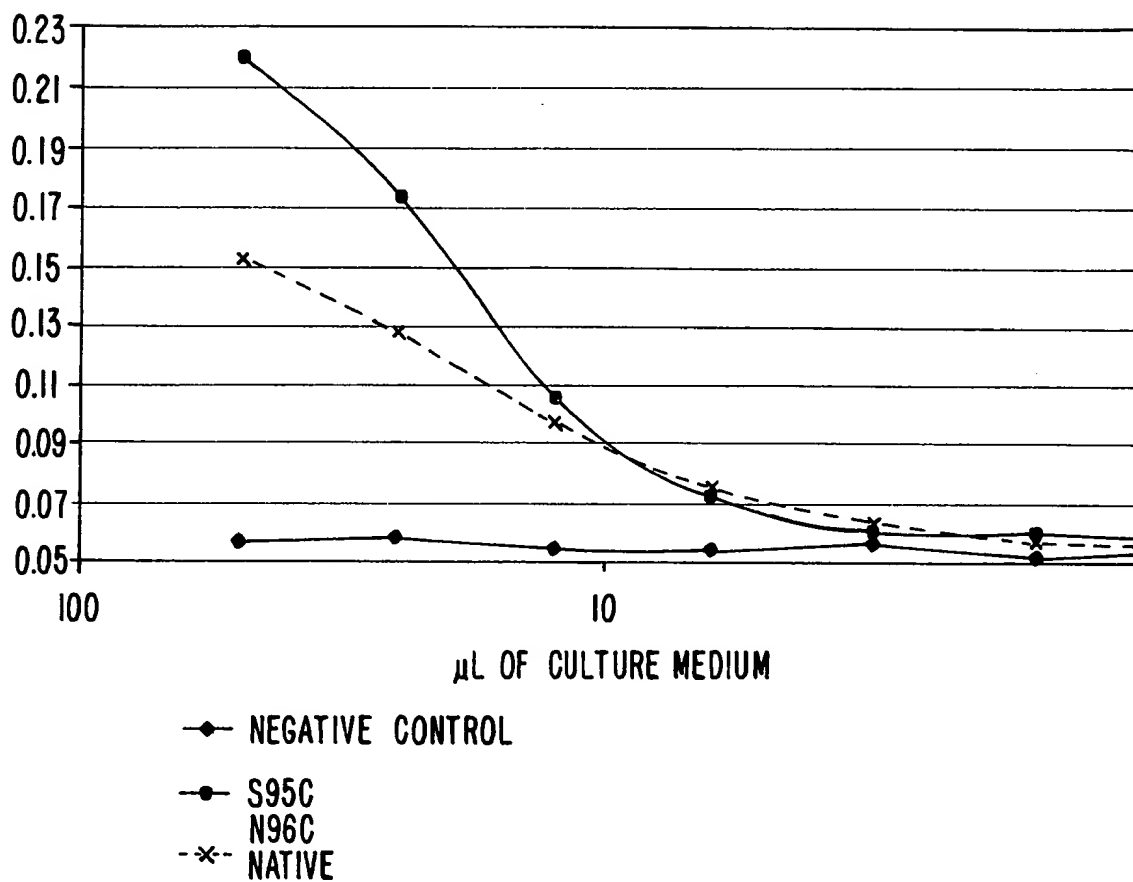


FIG. 16.

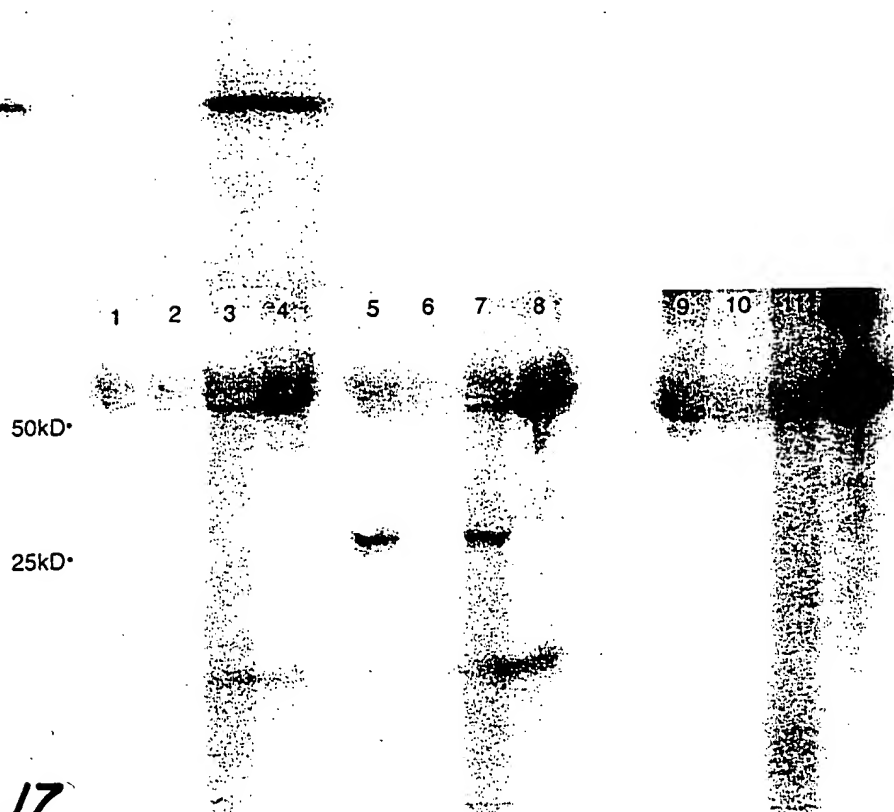


FIG. 17.



FIG. 18.

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